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Alcohol 48 (2014) 695–700

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

CETP TaqIB genotype modifies the association between alcohol and coronary heart disease: The INTERGENE case-control study

Kirsten Mehlig [a,*], Elisabeth Strandhagen [a], Per-Arne Svensson [b,c], Annika Rosengren [c], Kjell Torén [d], Dag S. Thelle [a,e], Lauren Lissner [a]

[a] Department of Public Health and Community Medicine, Institute of Medicine, University of Gothenburg, 405 30 Gothenburg, Sweden
[b] Sahlgrenska Center for Cardiovascular and Metabolic Research, University of Gothenburg, 413 45 Gothenburg, Sweden
[c] Department of Molecular and Clinical Medicine, Institute of Medicine, University of Gothenburg, 413 45 Gothenburg, Sweden
[d] Section of Occupational and Environmental Medicine, Institute of Medicine, University of Gothenburg, 405 30 Gothenburg, Sweden
[e] Department of Biostatistics, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, 0317 Oslo, Norway

Keywords:
Coronary heart disease
Alcohol
CETP polymorphism
Gene-environment interaction
Prevented fraction

A B S T R A C T

Alcohol consumption at moderate levels has been associated with decreased risk of coronary heart disease (CHD). However, the cardio-protective effect of alcohol may be restricted to subjects with a particular genotype of the cholesteryl ester transfer protein (CETP) polymorphism. There is evidence for this from one study in men, but the finding has not been confirmed since. The present study specifically re-examines the potential modification of the association between alcohol consumption and CHD by the *CETP* TaqIB (rs708272) polymorphism in a sample including both men and women. The INTERGENE case-control study consists of 618 patients with CHD and 2921 control subjects, of whom 19% were homozygous for the *CETP* TaqIB B2 allele. Alcohol consumption was categorized into sex-specific tertiles of ethanol intake, with non-drinkers constituting a separate category. Logistic regression was used to determine the association between CHD with genotype, ethanol intake, and their interaction. Participants with intermediate ethanol intake (2nd tertile) had lower risk of CHD than those with low ethanol intake (odds ratio [OR] = 0.65; 95% confidence interval [CI] 0.50–0.85). The strongest protective association was seen in the *CETP* TaqIB B2 homozygotes for intermediate vs. low ethanol intake (odds ratio OR = 0.21; 95% CI 0.10–0.44). The interaction between ethanol intake and genotype was statistically significant ($p = 0.008$), and of similar size in men and women though significant only in men ($p = 0.01$). The effect modification could not be explained by differences in lifestyle, socioeconomics, or alcohol-related biological variables such as HDL–cholesterol. Our study is the first to replicate previous findings of an effect modification in men. It gives only suggestive results for women, possibly due to the small number of female cases ($n = 165$). The prevented fraction for the favorable combination of genotype and alcohol consumption is about 6%, a value suggesting that the cardio-protective effect of moderate alcohol consumption applies only to a small segment of the general population.
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Introduction

Alcohol consumption at a so-called "moderate" level, up to 1 drink/day for women and 2 for men (1 drink corresponding to 14 g or 0.6 ounces of ethanol), has been associated with a decreased risk of coronary heart disease (CHD) (Doll, Peto, Boreham, & Sutherland, 2005; Mäkelä, Valkonen, & Poikolainen, 1997; Mukamal & Rimm, 2001; O'Keefe, Bybee, & Lavie, 2007; U.S. Dept. of Agriculture and U.S. Dept. of Health and Human Services, 2005). The consistency of the association suggests that there might be a causal relationship, and a number of pathways including antioxidant effects of polyphenols in wine (Opie & Lecour, 2007), and the more general high density lipoprotein-cholesterol (HDL) raising effect of alcohol *per se* have been suggested (Lewis, 2006). The latter would be via the role of HDL in the reverse transport of cholesterol from peripheral arteries to the liver, which has a CHD risk-reducing effect and is partly regulated by the glycoprotein cholesteryl ester transfer protein (CETP). The activity is determined by the *CETP* gene, including the intronic TaqIB polymorphism (rs708272). In 1995, Fumeron et al. showed an interaction between the *CETP* TaqIB polymorphism and alcohol consumption on the risk for coronary heart disease in a

The authors declare that there are no conflicts of interest.

* Corresponding author: Department of Public Health and Community Medicine, Sahlgrenska Academy, University of Gothenburg, Box 454, SE – 405 30 Gothenburg, Sweden. Tel.: +46 31 786 6844; fax: +46 31 778 1704.
E-mail address: kirsten.mehlig@gu.se (K. Mehlig).

http://dx.doi.org/10.1016/j.alcohol.2014.08.011
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study including 1236 men (568 cases) from different regions in Northern Ireland and France (Fumeron et al., 1995). In B2 homozygotes, the odds ratio for myocardial infarction was reduced by ethanol intake of 50 g/day or more compared to lower or no intake (OR = 0.39; 95% CI 0.20–0.75), while a similar protective association was not observed for B1 carriers (p value for interaction = 0.034). Later studies on this topic gave conflicting results (Boekholdt et al., 2005; Corella et al., 2010; Jensen, Mukamal, Overvad, & Rimm, 2008) and none fully replicated the original result.

Varying effects of the *CETP* TaqIB polymorphism may be due to heterogenous populations and different impact of lifestyle habits, but the possibility that the cardio-protective effect of alcohol is restricted to a subset of the population with a certain *CETP* TaqIB genotype cannot be excluded. We therefore aimed to re-examine the association between alcohol consumption and coronary heart disease and its possible modification by the *CETP* TaqIB polymorphism in a population-based case-control study based in southwest Sweden. In contrast to many previous studies, we are able to use several ethanol exposure categories and study possible non-linear associations with CHD.

Materials and methods

Study population

The study is a population-based case-control study that is part of the INTERGENE research program assessing the INTERplay between GENEtic susceptibility, lifestyle, biological, and psychosocial factors for the risk of chronic diseases in southwest Sweden. The INTERGENE cohort comprises randomly sampled individuals from a source population aged 25–74 at the time of sampling on April 1, 2001. As of December 2004, 3614 members of the target population sample had been examined. In addition to the cohort, the study population includes surviving cases of acute coronary heart disease identified from the same source population as the cohort.

Case registration and definition

In parallel with the cohort examinations, patients below the age of 75 years admitted to 3 regional hospitals for acute coronary syndrome and diagnosed with myocardial infarction (ICD 10: I21.0–I21.9), with typical history, ECG, and enzyme changes or unstable angina (ICD 10: I20.0) were invited to the study while still in the ward. Six hundred eighteen patients (453 men and 165 women) were included in this study, of whom 209 men and 86 women had a first-time acute myocardial infarction or unstable angina while the remaining 323 had an exacerbation of previously diagnosed coronary heart disease. All the registered cases underwent similar procedures as the cohort members. For further information on the study population, recruitment, and participation see www.intergene.gu.se and previous publications (Berg et al., 2008; Strandhagen et al., 2010).

Study procedures

The examination included self-administered questionnaires on environmental and lifestyle-related exposure variables, anthropometric measures, and collection of venous blood samples. During examination, all participants were asked about the frequency of intake of different types of alcoholic beverage (low-alcohol beer, medium-strong beer, strong beer, wine, dessert wine, and spirits). Control subjects were asked about the previous 12 months before examination, and cases were asked about the previous 12 months before their most recent coronary event. For each type of alcohol, there were 8 response categories, ranging from never to 3 or more times per day. These self-reported frequencies of intake were combined with age and sex-dependent standard servings of alcoholic beverage consumed per occasion to calculate the total consumption of ethanol in g/day (Berg et al., 2009). In addition, the following health, lifestyle, and socioeconomic variables were recorded and analyzed as dichotomous factors: diabetes status, use of lipid-lowering drugs, smoking status (ever vs. never), leisure time physical activity (at least 4 h/week vs. less), level of education (university vs. less), being married or living with a partner (yes vs. no), and having financial difficulties (yes vs. no).

Laboratory analysis

Participants fasted at least 4 h before blood samples were drawn. For the coronary patients, blood samples were drawn after the event. The blood samples were collected into tubes containing 0.1% EDTA for immediate serum lipid and glucose analysis. Serum total cholesterol and triglyceride concentrations were determined using enzymatic assays. Serum HDL-cholesterol concentrations were measured after dextran sulfate magnesium precipitation of apoB-containing lipoproteins. Whole blood was stored at −70 °C in 1.5 mL aliquots for DNA extraction.

Genotyping

The TaqIB polymorphism (rs708272) is located in the first intron of the *CETP* gene. The genotyping was performed using the 7900HT sequence detection system and the genotyping assay C_9615318_10 (Applied Biosystems, Foster City, CA). Each reaction consisted of 1.25 μL of 20X C_9615318_10 genotyping assay mix, 12.5 μL TaqMan Universal PCR Master Mix, 10.25 μL RNase-free water, and 1 μL of sample DNA (10–15 ng). The PCR reaction was initially heated for 10 min at 95 °C, followed by 40 cycles of 15 s at 92 °C and 1 min at 60 °C.

Statistical methods

Differences in means of continuous variables or proportions were tested using t test (for age) or age-adjusted linear and logistic regression, stratified by sex. The values of BMI, triglycerides, HDL-cholesterol, and ethanol intake (current abstainers excluded) were log-transformed before analysis. Multiple logistic regression was used to describe the association between explanatory variables such as genotype and ethanol intake and their interaction with CHD. All models were adjusted for age, BMI, HDL-cholesterol (including quadratic terms), sex, and smoking status. Three categories of ethanol intake were defined using sex-specific cut-off values for tertiles of ethanol intake in the male and the female cohort, while the group of non-users of alcohol defined a 4th group. Because abstainers constitute a heterogenous group ranging from former alcohol abuse to life-long abstention, the tertile of consumers with lowest alcohol consumption was chosen as a reference category. The results are given in terms of odds-ratios (OR) and their 95% confidence intervals in parentheses. The analysis was repeated for first-time patients (n = 295) only, excluding patients with a history of cardiovascular disease. We also retested the association between ethanol, genotype, and CHD based on tertiles of ethanol intake per kg body weight as an alternative way to adjust for the confounding effect of body weight. In a *post hoc* analysis, we tested for differences in leisure time physical activity (at least 4 h/week vs. less), education (university vs. less), being married or living with a partner (yes vs. no), financial insecurity (yes vs. no), as well as diabetes status (yes vs. no) and triglyceride level between the 2 genotype groups B2B2 vs. not B2B2 using the age-adjusted regression methods. The statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA.). Statistical significance was set at p value $\leq$0.05 (2 sided test).

Prevented fraction

The prevented fraction of a disease related to a particular indicator of protection is the fraction of cases in the population that is prevented by the marker of protection and/or factors associated with it. This is a theoretical concept which can be based on relative risk or odds ratio as an approximation of the former (Miettinen, 1974). The theoretical prevented fraction (PF) of CHD cases in the general population avoided by the favorable factor (here, a specific combination of genotype and ethanol intake) is calculated as $PF = P \times (1 - OR)$ where the prevalence of the factor (P) is estimated in the cohort subjects and OR denotes the odds ratio for CHD in subjects with the favorable factor compared to those without (Gargiullo, Rothenberg, & Wilson, 1995).

Ethics statement

All subjects gave their written consent to the study, and the protocol was approved by the Regional ethics review board, Forskningsetikkommité (no. Ö 237/2000). The study complies with the Declaration of Helsinki.

Results

Description of CHD cases and controls

Descriptive properties of CHD cases and control subjects are shown in Table 1. A lower percentage of alcohol users and a lower median level of consumption among users were seen in coronary patients of both sexes. The distribution of ethanol intake among users was strongly skewed to the right, with a larger variation of intake among the cases compared with the controls. The age-adjusted difference by case status was also observed with respect to ethanol intake per kg body weight. Due to the lower alcohol consumption in women (cases and/or controls) compared to men, this variable was categorized using sex-specific percentiles derived from the cohort, to allow for an analysis combining both sexes. Among current consumers, cut-off values for tertiles of ethanol intake are given by 3.2 and 6.3 for women, and 6.5 and 13.1 for men (in g/day). As described in the Materials and methods section, the intakes among cases refer to 1 year before the event, and in controls to 1 year before examination.

The distribution of genotype was similar in both female and male cases and controls, and did not deviate from Hardy–Weinberg equilibrium. The prevalence of the B2 allele in the cohort did not depend on age (data not shown).

At the time of the examination, male and female CHD cases were on average 10 and 12 years older than the respective control subjects and had a higher age-adjusted BMI. CHD cases had lower levels of HDL-cholesterol, whereas triglyceride levels were higher in male patients compared to controls. The proportion of ever-smoking, self-reported diabetes and use of cholesterol-lowering drugs was higher among coronary patients for both sexes. Patients were also less likely to report leisure time physical activity. Higher education was related with case status in women, while living alone was associated with CHD in men. Patients of both sexes reported financial difficulties more often than controls.

Risk for CHD by CETP TaqIB polymorphism

The B2B2 genotype was associated with a lower CHD risk, adjusted for age, sex, body mass index (BMI), and smoking status [OR = 0.71 (0.53–0.94)], with a similar magnitude in men [OR = 0.74 (0.53–1.05)] and women [OR = 0.62 (0.37–1.07)]. The effect of B2B2 relative to B1B1 was no longer observed when further adjusted for HDL-cholesterol (Table 2A). Since there was no risk difference between B1B2 and B1B1 genotypes, we restricted subsequent analyses to the comparison between B2 homozygotes and B1B2 combined with B1B1.

Risk for CHD by alcohol consumption and modification of risk by CETP TaqIB polymorphism

Table 2B shows the odds ratio for CHD by category of ethanol intake, adjusted for age, sex, smoking status, BMI, and HDL-cholesterol. The group with intermediate ethanol intake had a lower risk compared to low intake, while there was no association with CHD for abstainers and high consumers.

Table 2C shows the results of the joint model of CHD for ethanol intake and genotype including their interaction, presented in terms

Table 1
Properties of patients and controls from the INTERGENE case-control study, stratified by sex. Mean value (SD) for continuous variables (except ethanol intake), counts (%) for categorical variables, *p* value for age-adjusted test for comparison by case status.

	Women		Men	
	Cases	Controls	Cases	Controls
	$n = 165$	$n = 1543$	$n = 453$	$n = 1378$
Users of alcohol	132 (80%)	1346 (87%)	402 (89%)	1283 (93%)
Ethanol intake (g/day)[a,b]	3.3 (1.6–7.4)*	4.7 (2.7–7.7)	9.0 (4.3–16.4)*	9.7 (5.6–15.6)
Ethanol intake/body weight (g/day/kg)[a,b]	0.04 (0.03–0.09)**	0.07 (0.04–0.11)	0.10 (0.05–0.20)*	0.12 (0.07–0.19)
CETP genotype: B1B1	58 (35.2%)	517 (33.5%)	151 (33.3%)	421 (30.5%)
B1B2	84 (50.9%)	730 (47.3%)	229 (50.6%)	690 (50.1%)
B2B2	23 (13.9%)	296 (19.2%)	73 (16.1%)	267 (19.4%)
Age (years)	62.7 (8.1)*	50.5 (13.6)	61.4 (8.4)***	51.1 (13.4)
Body mass index (kg/m^2)[a]	28.2 (4.8)**	25.5 (4.3)	27.7 (4.0)***	26.6 (3.6)
HDL-cholesterol (mmol/L)[a]	1.56 (0.43)***	1.78 (0.45)	1.28 (0.34)***	1.46 (0.38)
Triglycerides (mmol/L)[a]	1.65 (1.13)	1.21 (0.67)	1.71 (1.30)***	1.57 (1.09)
Ever smoking	100 (61%)***	756 (49%)	352 (78%)***	705 (51%)
History of diabetes	30 (18.2%)***	40 (2.6%)	78 (17.2%)***	61 (4.4%)
Cholesterol lowering drugs	117 (70.8%)***	81 (5.3%)	357 (78.8%)***	94 (6.8%)
Leisure time physical activity (≥4 h/week)	132 (80%)***	1411 (92%)	382 (84%)***	1211 (88%)
Higher education	140 (85%)*	1004 (65%)	366 (81%)	999 (73%)
Being married/cohabitate	107 (65%)	1120 (73%)	329 (73%)***	1078 (79%)
Financial insecurity	39 (24%)**	316 (21%)	82 (18%)**	226 (17%)

p values: *<0.05, **<0.01, ***<0.001.
[a] Log-transformed before analysis.
[b] Alcohol consumers only, median (1st quartile – 3rd quartile).

Table 2
Odds ratio of CHD (95% CI) for *CETP* TaqIB genotype and ethanol intake. Separate models for genotype (A) and ethanol intake (B), respectively, and the results from a joint model of genotype, ethanol intake, and their interaction (C), all regressions adjusted for age, sex, smoking status, BMI, and HDL-cholesterol.

A: Main effect of genotype				
		B1B1	B1B2	B2B2
		1(ref)	1.03 (0.83–1.29)	0.89 (0.66–1.20)
B: Main effect of ethanol intake[a]				
	Abstainer	Low	Intermediate	High
	1.06 (0.76–1.47)	1 (ref)	0.65 (0.50–0.85)**	0.90 (0.70–1.17)
C: Joint effect of genotype and ethanol intake[a]				
	Abstainer	Low	Intermediate	High
B1B1 + B1B2[b]	1.12 (0.77–1.62)	1 (ref)	0.80 (0.59–1.06)	1.03 (0.77–1.36)
B2B2[b]	0.76 (0.36–1.64)	1 (ref)	0.21 (0.10–0.44)***	0.48 (0.26–0.88)*
Interaction[c]	0.68 (0.29–1.59)	1 (ref)	0.27 (0.12–0.59)**	0.46 (0.24–0.91)*

p values: *<0.05, **<0.01, ***<0.001.
[a] Low: ♀ < 3.2, ♂ < 6.5, intermediate: ♀ 3.2–6.3, ♂ 6.5–13.1, high: ♀ > 6.3, ♂ > 13.1 (g/day).
[b] OR for ethanol intake (ref = low intake) by genotype category.
[c] OR for the product term ethanol intake × genotype.

of odds ratios of CHD for the categories of ethanol intake stratified by genotype category (line 1 & 2). Again, the group with intermediate ethanol intake had the lowest risk compared to low intake, but this was markedly more pronounced in B2 homozygotes than in B1 carriers. A negative association with risk of CHD was also observed for high vs. low intake in B2 homozygotes, but not in B1 carriers or in the full sample. The last row of Table 2C shows a significant effect modification of intermediate or high ethanol intake by *CETP* B2B2 genotype, compared to low intake. The overall *p* value for interaction was equal to 0.008. The OR for CHD in abstainers did not differ significantly from the risk in subjects with low ethanol intake in either stratum, and the effect modification was not changed when current abstainers were chosen as reference. The cardio-protection at intermediate ethanol intake was also observed in men and women separately [OR = 0.42 (0.20–0.88) for B2B2 vs. non B2B2 in men with intermediate intake, and OR = 0.34 (0.08–1.52) for women with intermediate intake]. However, this was significant in men only (overall *p* value for interaction = 0.01 for men, 0.2 for women). The corresponding OR for both sexes combined was OR = 0.41 (0.22–0.79).

Fig. 1 summarizes the results of the separate model for ethanol intake (1A) and the joint model for ethanol intake, *CETP* B2B2 genotype, and their interaction (1B & 1C). The inverse association of intermediate ethanol intake with risk of CHD observed in the full sample is mainly due to a strong effect in the B2 homozygotes, which constitute 19% of the sample. Only in the latter category is it observed that high ethanol intake has a smaller risk than low ethanol intake.

Sensitivity analyses

A similar U shaped risk curve among B2 homozygotes was observed when alcohol consumption was categorized into quartiles (and abstainers) or when alcohol consumption was based on tertiles of ethanol intake per kg body weight (data not shown). The results given in Table 2 did not depend on the adjustment for HDL-cholesterol, nor did they change when an interaction between genotype and HDL-cholesterol was included (*p* value for interaction > 0.1, data not shown). There were no differences between the 2 genotype strata regarding leisure time physical activity, education, being married or living with a partner, financial insecurity, triglyceride level, or diabetes status, and the inclusion of these variables in the model did not reduce the effect modification reported in Table 2.

The protective effect of B2B2 genotype in the categories of intermediate or high ethanol intake was more pronounced when we restricted the cases to the 295 first-time coronary cases who would be less likely to have altered their alcohol intake due to previous events: OR = 0.13 (0.04–0.41) for intermediate vs. low intake in

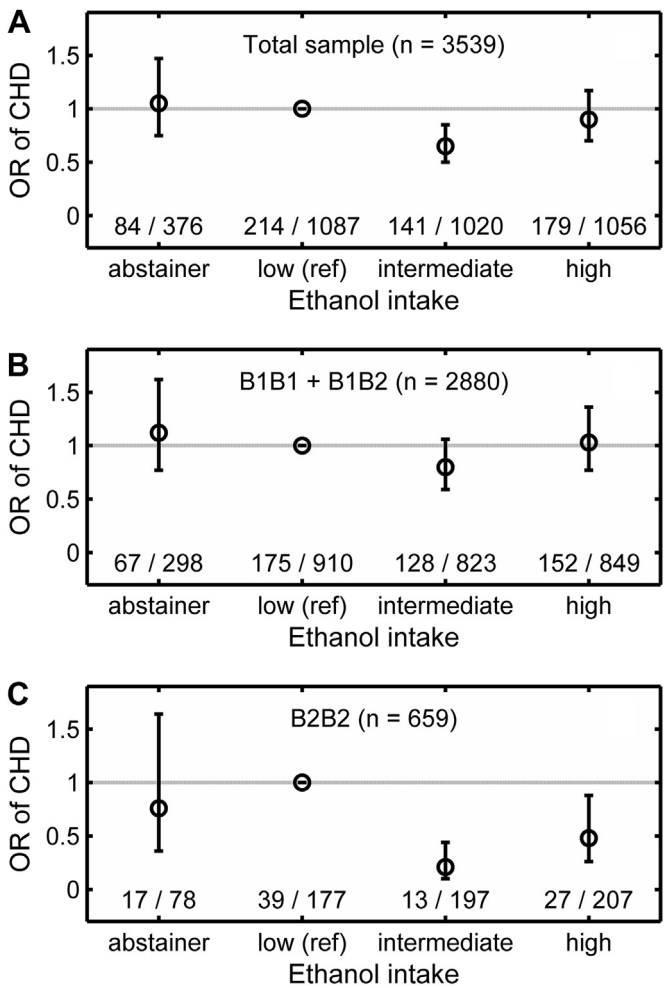

Fig. 1. Odds ratio and 95% CI for CHD by categories of ethanol intake and *CETP* TaqIB genotype. OR for CHD by categories of ethanol intake for all genotypes combined (A) and stratified by genotype (B, C), including the interaction between ethanol intake and genotype. Both models are adjusted for age, sex, smoking, BMI, and HDL. The lower part of each panel gives the number of cases/the total number of subjects in each category of ethanol intake.

B2B2, OR = 0.34 (0.15–0.80) for high vs. low intake in B2B2 and OR = 0.70 (0.48–1.03) for intermediate vs. low intake in B1 carriers, OR = 1.06 (0.75–1.52) for high vs. low intake in B1 carriers, overall p value for interaction = 0.015. Also, we observed the same interaction pattern as described in Table 2 when age was restricted to 60 years or above (373 cases, 856 controls, average age = 67 in both groups). In this subsample, the odds ratio of CHD for intermediate vs. low ethanol intake was given by OR = 0.16 (0.04, 0.62) in B2 homozygotes compared to OR = 0.78 (0.53, 1.15) in B1 allele carriers, p value for interaction = 0.03.

The cardio-protective effect of alcohol and CETP TaqIB polymorphism as a prevented fraction

In order to place this apparent gene-environment interaction in a public health context, we estimated the fraction of coronary cases in the population that may have been prevented by intermediate or high alcohol consumption among the B2B2 carriers. First, we calculated the prevalence (P) of the combination of B2 homozygosity and intermediate or high ethanol intake, which was given by 0.125 in the cohort-based control subjects. This, together with the odds ratio for CHD [OR = 0.49 (95% CI 0.35–0.68) yielded a PF of 0.06 (0.04–0.09)], indicates that the frequency of CHD disease would have been increased by roughly 6% if this combination of alcohol consumption and B2B2 genotype had not existed in the population. The implications of this relatively low estimate of prevented cases are discussed in more detail below.

Discussion

In this population-based study, the overall observation of a reduced risk for CHD in subjects with intermediate or high alcohol consumption (≥3.2 g/day for women and ≥6.5 g/day for men) was most strongly observed in *CETP* TaqIB B2 homozygotes. This modification of the effect of ethanol intake by genotype was of similar size in both men and women in spite of their different level of alcohol consumption. However, the effect modification was statistically significant in men only, the result in women probably being limited by sample size because only 27% of 618 CHD cases were female. The cardio-protective effect of alcohol in the *CETP* TaqIB B2 homozygotes could not be explained by a mediating effect of HDL-cholesterol or an interaction between HDL-cholesterol and genotype, nor was the effect reduced after adjustment for different socioeconomic and lifestyle variables, or explained by selective survival of certain genotype groups. The finding that the protective effect of alcohol is not due to HDL-cholesterol is consistent with the results in a recent Norwegian study (Magnus et al., 2011). Our results that the reduced risk is associated with intermediate and high consumption in a certain *CETP* genotype strengthens the notion that there is a direct effect of alcohol intake on the risk of CHD and is not due to confounding factors or clustering of healthy habits.

These results confirm the first report on the association between *CETP* genotype, alcohol, and CHD, a case-control study on myocardial infarction comprising men in Northern Ireland and 3 areas in France (ECTIM) (Corbex et al., 2000; Fumeron et al., 1995). This 1995 study showed decreasing CHD risk with increasing ethanol intake in B2 homozygotes compared to the other genotypes, which was observed for an ethanol intake larger or equal to 50 g/day and was strongest among the heavy drinkers (≥75 g per day). Our study is the first to replicate this interaction effect in men; however, there was a much lower level of alcohol intake and a U shaped risk profile with respect to ethanol intake. Our result for women is also similar to results from the Nurses' Health Study, where a 60% risk reduction was found for female B2 carriers consuming at least 5 g/day compared to none, and no effect of alcohol in B1 homozygotes (Jensen et al., 2008), but the interaction was not significant either. Lack of an interaction effect in both the female and the corresponding male cohort studied by Jensen et al. (2008) could be explained by the smaller sample size and the comparison between B2 carriers and B1 homozygotes.

Finally, our results can be contrasted to a meta-analysis of 7 studies including ECTIM (Fumeron et al., 1995) that found no significant interaction between *CETP* TaqIB and ethanol on CHD (Boekholdt et al., 2005). The lack of a convincing effect may be explained by the fact that a dichotomized alcohol variable had to be used comparing current use of alcohol to abstinence when summarizing the results of the studies. The division into drinking/no drinking is obviously too crude to identify the non-linear association between ethanol intake and CHD. This could also be the explanation for the adverse association between *CETP* TaqIB B2B2 genotype and CHD in drinkers reported in a more recent study from Spain (Corella et al., 2010).

A strength of this study is that we use several levels of ethanol intake in addition to abstinence even though the exposure is based on self-reported usual alcohol consumption, which is prone to many types of error. Under-reporting of alcohol consumption is a known problem, and might explain part of the discrepancy with other studies regarding the absolute amount of alcohol intake. However, the positive correlation between HDL-cholesterol and ethanol intake in a previous study of the cohort (Tognon et al., 2012) supports the validity of the latter. Among patients, there could be under-reporting due to knowledge of disease or truly reduced alcohol consumption after the event; however, coronary patients in Sweden are not usually advised to change their alcohol consumption. The main result of this study, the protective effect of intermediate alcohol consumption in B2 homozygotes compared to non-B2B2 carriers (Fig. 1), is robust in the sense that misclassification of ethanol intake should be independent of genotype. Still we cannot exclude the possibility that the subgroup of intermediate ('moderate') users of alcohol displays a clustering of generally healthier lifestyle variables, which act together in the protection against CHD. When retested adding leisure time physical activity, financial security, educational level, marital status, and diabetes status as covariates, no change in the effect of alcohol consumption and genotype on CHD was observed, nor did we see differences in these variables comparing the favorable combination of intermediate or high ethanol intake and B2B2 genotype with the rest of the study population.

The cardio-protective effect of alcohol – not for everyone?

The common attitude today is that moderate alcohol intake will decrease everyone's risk of coronary heart disease. Together with the ECTIM study (Fumeron et al., 1995), the present study suggests that this message may be too general and should be assessed in light of the weak overall effect of alcohol on CHD in the general population and the emerging knowledge about genetic susceptibility. Future research should re-assess the magnitude of the prevented fraction in the general population, using data on past and current alcohol consumption and incident cases of CHD.

Acknowledgments

The study was funded by the Swedish Research Council [VR 521-2010-2984], the Swedish Heart-Lung Foundation [2009-0390], and the Swedish council for working life and social research [Epilife 2006-1506].

References

Berg, C., Lappas, G., Wolk, A., Strandhagen, E., Torén, K., Rosengren, A., et al. (2009). Eating patterns and portion size associated with obesity in a Swedish population. *Appetite, 52*, 21–26.

Berg, C. M., Lappas, G., Strandhagen, E., Wolk, A., Torén, K., Rosengren, A., et al. (2008). Food patterns and cardiovascular disease risk factors: the Swedish INTERGENE research program. *The American Journal of Clinical Nutrition, 88*, 289–297.

Boekholdt, S. M., Sacks, F. M., Jukema, J. W., Shepherd, J., Freeman, D. J., McMahon, A. D., et al. (2005). Cholesteryl ester transfer protein TaqIB variant, high-density lipoprotein cholesterol levels, cardiovascular risk, and efficacy of pravastatin treatment: individual patient meta-analysis of 13,677 subjects. *Circulation, 111*, 278–287.

Corbex, M., Poirier, O., Fumeron, F., Betoulle, D., Evans, A., Ruidavets, J. B., et al. (2000). Extensive association analysis between the CETP gene and coronary heart disease phenotypes reveals several putative functional polymorphisms and gene-environment interaction. *Genetic Epidemiology, 19*, 64–80.

Corella, D., Carrasco, P., Amiano, P., Arriola, L., Chirlaque, M. D., Huerta, J. M., et al. (2010). Common cholesteryl ester transfer protein gene variation related to high-density lipoprotein cholesterol is not associated with decreased coronary heart disease risk after a 10-year follow-up in a Mediterranean cohort: modulation by alcohol consumption. *Atherosclerosis, 211*, 531–538.

Doll, R., Peto, R., Boreham, J., & Sutherland, L. (2005). Mortality in relation to alcohol consumption: a prospective study among male British doctors. *International Journal of Epidemiology, 34*, 199–204.

Fumeron, F., Betoulle, D., Luc, G., Behague, I., Ricard, S., Poirier, O., et al. (1995). Alcohol intake modulates the effect of a polymorphism of the cholesteryl ester transfer protein gene on plasma high density lipoprotein and the risk of myocardial infarction. *The Journal of Clinical Investigation, 96*, 1664–1671.

Gargiullo, P. M., Rothenberg, R. B., & Wilson, H. G. (1995). Confidence intervals, hypothesis tests, and sample sizes for the prevented fraction in cross-sectional studies. *Statistics in Medicine, 14*, 51–72.

Jensen, M. K., Mukamal, K. J., Overvad, K., & Rimm, E. B. (2008). Alcohol consumption, TaqIB polymorphism of cholesteryl ester transfer protein, high-density lipoprotein cholesterol, and risk of coronary heart disease in men and women. *European Heart Journal, 29*, 104–112.

Lewis, G. F. (2006). Determinants of plasma HDL concentrations and reverse cholesterol transport. *Current Opinion in Cardiology, 21*, 345–352.

Magnus, P., Bakke, E., Hoff, D. A., Høiseth, G., Graff-Iversen, S., Knudsen, G. P., et al. (2011). Controlling for high-density lipoprotein cholesterol does not affect the magnitude of the relationship between alcohol and coronary heart disease. *Circulation, 124*, 2296–2302.

Mäkelä, P., Valkonen, T., & Poikolainen, K. (1997). Estimated numbers of deaths from coronary heart disease "caused" and "prevented" by alcohol: an example from Finland. *Journal of Studies on Alcohol, 58*, 455–463.

Miettinen, O. S. (1974). Proportion of disease caused or prevented by a given exposure, trait or intervention. *American Journal of Epidemiology, 99*, 325–332.

Mukamal, K. J., & Rimm, E. B. (2001). Alcohol's effects on the risk for coronary heart disease. *Alcohol Research & Health, 25*, 255–261.

O'Keefe, J. H., Bybee, K. A., & Lavie, C. J. (2007). Alcohol and cardiovascular health: the razor-sharp double-edged sword. *Journal of the American College of Cardiology, 50*, 1009–1014.

Opie, L. H., & Lecour, S. (2007). The red wine hypothesis: from concepts to protective signalling molecules. *European Heart Journal, 28*, 1683–1693.

Strandhagen, E., Berg, C., Lissner, L., Nunez, L., Rosengren, A., Torén, K., et al. (2010). Selection bias in a population survey with registry linkage: potential effect on socioeconomic gradient in cardiovascular risk. *European Journal of Epidemiology, 25*, 163–172.

Tognon, G., Berg, C., Mehlig, K., Thelle, D., Strandhagen, E., Gustavsson, J., et al. (2012). Comparison of apolipoprotein (apoB/apoA-I) and lipoprotein (total cholesterol/HDL) ratio determinants. Focus on obesity, diet and alcohol intake. *PLoS One, 7*, e40878.

U.S. Dept. of Agriculture and U.S. Dept. of Health and Human Services. (2005). *Nutrition and Your Health: Dietary Guidelines for Americans*.